BEST AVAILABLE IMAGES

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images may include (but are not limited to):

- BLACK BORDERS
- TEXT CUT OFF AT TOP, BOTTOM OR SIDES
- FADED TEXT
- ILLEGIBLE TEXT
- SKEWED/SLANTED IMAGES
- COLORED PHOTOS
- BLACK OR VERY BLACK AND WHITE DARK PHOTOS
- GRAY SCALE DOCUMENTS

IMAGES ARE BEST AVAILABLE COPY.

As rescanning documents *will not* correct images, please do not report the images to the Image Problem Mailbox.

PCT/AU00/00873
10/031492
REC'D 04 AUG 2000
WIPO PCT

Patent Office
Canberra

AU 00/00873

I, LEANNE MYNOTT, ACTING MANAGER PATENT ADMINISTRATION hereby certify that annexed is a true copy of the Provisional specification in connection with Application No. PQ 1719 for a patent by WESTMEAD HOSPITAL and THE UNIVERSITY OF SYDNEY filed on 20 July 1999.

EJKW

WITNESS my hand this
Thirty-first day of July 2000

L. Mynott

LEANNE MYNOTT
ACTING MANAGER PATENT
ADMINISTRATION

PRIORITY DOCUMENT
SUBMITTED OR TRANSMITTED IN COMPLIANCE WITH RULE 17.1(a) OR (b)

AUSTRALIA

Patents Act 1990

WESTMEAD HOSPITAL,

THE UNIVERSITY OF SYDNEY

PROVISIONAL SPECIFICATION

Invention Title:

Neurotropic virus transport

The invention is described in the following statement:

Technical Field

The present invention relates to viral infections, particularly cellular and tissue interactions during infection.

Background Art

Little is known about the mechanisms of transport of neurotropic viruses, such as Herpes simplex virus (HSV), varicella-zoster virus and rabies, within neurones. For these viruses, which replicate in the nucleus, anterograde transport from the cell body of dorsal root human ganglionic (DRG) neurons to the axon terminus occurs over long distances. In the case of HSV, there is increasing evidence for separate, fast anterograde axonal transport of viral nucleocapsids coated with tegument along microtubules (MTs) and of glycoproteins in axonal transport vesicles. An understanding of the mechanisms of transport of these viruses would be of benefit. Once this information is obtained, means to interrupt or prevent this transport would be a good candidate for antiviral therapy.

The present inventors have now obtained useful evidence for the direct interaction between a viral structural tegument protein and an ubiquitous cellular protein.

Disclosure of Invention

In a first aspect, the present invention consists in a method of preventing or reducing transport of a neurotropic virus within a neuron, the method comprising altering or preventing interaction between a structural tegument protein of the virus and a motor protein in the neuron such that virus transport in the neuron is prevented or reduced.

In a preferred embodiment, the neurotropic virus is Herpes simplex virus (HSV), varicella-zoster virus, or rabies virus. More preferably, the virus is HSV, the structural tegument protein is US11, and the motor protein is kinesin.

The method according to the present invention can be carried out by providing a motor protein-like molecule to the neuron infected by the virus such that any virus present will bind the motor protein-like molecule via the structural tegument protein thereby preventing the normal interaction of the virus with cellular motor protein.

The motor protein-like molecule can be any suitable molecule and would include mimics of the motor protein or a parts of the motor protein to which the structural tegument protein binds or specifically interacts.

When the motor protein is kinesin in the case of HSV, the kinesin-like molecule can be any suitable molecule and would include mimics of kinesin or a parts of kinesin to which the structural tegument protein US11 of HSV binds or specifically interacts.

Knowing how these types of viruses move in neurons to infect neurons other cells and ultimately form lesions is an important advance and is useful for the development of antiviral strategies. Usually, during the latent state these viruses do not cause any real problems to an individual. It is during the active phase of the virus where clinical manifestations occur and suffering is caused. Preventing the actual transport of the virus down infected neurons should at least combat or prevent the clinical symptoms of re-infection. Similarly, preventing movement up the neuron may prevent the formation of latent state of the virus in ganglia or at least reduce the incidence of re-occurrence of manifestations of the disease in infected individuals.

In a second aspect, the present invention consists in a modified neurotropic virus which has lost or has reduced ability to be transported in neurons, the virus comprising a mutation in a tegument protein such that the mutated tegument protein has abnormal interaction with a cellular motor protein.

In a third aspect, the present invention consists in the use of a compound to prevent or reduce the interaction between a neurotropic viral tegument protein with a cellular motor protein to prevent or reduce viral transport in a neuron.

In a preferred embodiment of the second and third aspects of the present invention, the neurotropic virus is Herpes simplex virus (HSV), varicella-zoster virus, or rabies virus. More preferably, the virus is HSV, the structural tegument protein is US11, and the motor protein is kinesin.

This study raises many questions about the mechanisms of tegument formation, location of US11 in the tegument of HSV, the potential role of motor proteins other than kinesin(s) and homologous interactions mediating the transport of other neurotropic viruses. Studies to map minimal binding regions of US11 and uKHC and identification of other cellular factors which facilitate or contribute to the interaction will be useful. Inhibition of this interaction by peptides and/or peptidomimetic analogues could provide a new strategy for antiviral treatment for this and other neurotropic viruses.

Incorporation of deletions into specifically attenuated live HSV (or other attenuated live neurotropic viruses) vaccine candidates could also be useful to prevent clinical recrudescence.

Throughout this specification, unless the context requires otherwise, the word "comprise", or variations such as "comprises" or "comprising", will be understood to imply the inclusion of a stated element, integer or step, or group of elements, integers or steps, but not the exclusion of any other element, integer or step, or group of elements, integers or steps.

In order that the present invention may be more clearly understood, preferred forms will be described with reference to the following examples and drawings.

Brief Description of Drawings

Figure 1. Summary of kinesin heavy chain structure and fusion proteins expressed in bacteria. a, Diagram of domain structure of ubiquitous kinesin heavy chain (uKHC). b, Diagram of fragments of uKHC expressed in bacteria. c, Oligo-histidine (His)-tagged (left) and MBP-tagged (right) proteins eluted from nickel-activated beads or amylose resin respectively and stained with coomassie blue.

Figure 2. Identification of HSV proteins which bind to uKHC. Complexes of His-uKHC and viral proteins eluted from nickel-activated beads were separated by SDS-PAGE and immunoblotted with a, anti-HSV1; b, monoclonal anti-VP16; or c, monoclonal anti-US11. Addition of mock or virus-infected Hep-2 cell lysates to His-KHC fragments is indicated (+). In each immunoblot the first two lanes, mock and virus-infected Hep-2 cell lysates are controls for antibody specificity.

Figure 3. Analysis of HSV-kinesin protein interactions using recombinant viral proteins. Protein complexes were separated by SDS-PAGE and immunoblotted with the indicated mouse monoclonal antibodies. a, Untagged VP16; or b, untagged US11 in bacterial lysates were incubated with His-tagged and MBP-tagged fusion constructs. Expression of untagged VP16 (a) and untagged US11 (b) in bacterial lysates was confirmed in the left-most lanes. c, Native VP16 from HSV1-infected Hep-2 cells or recombinant untagged VP16 was incubated with His-US11 or His-KHCstalk.

Figure 4. Transport of structural HSV antigens of a US11 deletion mutant and controls into axons of infected dissociated rat neonatal neurons *in vitro*. Similar experiments with human fetal DRG neurons showed the

same results. Dissociated DRG neurons were infected with control HSV1 (the clinical CW1 strain), the HSV1 US11 deletion mutant (R6604), and its rescuant (R6606) at 20 pfu/neuron. The appearance and transport into the principle axon of capsid (VP5), tegument (US11) and envelope glycoprotein (gC) antigens was followed by serial fixation over 6 to 36 h, by immunofluorescence and confocal microscopy [ ]. As shown previously, gC first appeared in axons at 13 hours and VP16 and VP5 at 17 hours. A=axon and N=nucleus. a-b, US11 was present in axons 24 h post infection (hpi) with HSV1 strain CW1 in the absence (a) and presence (b) of BFA. c-d, VP16 was present in axons 24 hpi in the absence (c) but not in the presence of BFA (d). Inset: Bright field microscopy shows intact axon (A). e-h, HSV1 antigens in the axon 24 hpi with either the HSV1 US11 deletion mutant or control rescuant. VP5 was not detected in axons infected with mutant (e) but was present in axons infected with control rescuant (f). (The stained axon disappears distally out of the plane). Inset in e shows the intact axon (A). gC was detected in axons whether infected with mutant (g) or control rescuant (h). Insets in d and e show the unstained axons by phase contrast microscopy.

Figure 5. Ultrastructural distribution of enveloped and unenveloped nucleocapsids of an HSV US11 deletion mutant (R6604) and control rescuant (R6606) late (26 hours) after infection of dissociated rat DRG neurons. The same results were observed with infected dissociated human fetal DRG neurons. Panel A shows unenveloped (arrow) and enveloped (arrow head) nucleocapsids adjacent to the Golgi within the cytoplasm of the cell body. Panel B shows enveloped virions (arrow) in the extracellular space adjacent to the cell body.

Modes for Carrying Out the Invention

METHODS

Cloning

The generation of the N-terminal oligohistidine-tagged uKHC constructs 555-772, 771-963, 771-876, 555-876, 555-813 and 855-963 (uKHC amino acid numbering) along with untagged human KLC (residues 4-569) are described elsewhere [1]. To express MBP fusions, a *Bam*HI fragment containing cDNA corresponding to uKHC771-963 [1] or KLC4-659 was inserted into pMAL-c2X. In the case of KLC, the *Nco*I (Klenow filled-

in)/*Eco*RI cDNA fragment containing KLC4-569 from pET-28a/KLC [1] was first inserted into *Bam*HI (Klenow filled-in)/*Eco*RI digested pET-28a.

Plasmid pRB4766 [2] containing HSV1 US11 genomic DNA in pGEX-KG was provided by B. Roizman. An untagged US11 construct was generated by digestion of pRB4766 with *Nco*I and insertion into the *Nco*I site of pET-28a. This results in an additional five amino acids (MGRLE) at the N-terminus of US11. Oligohistidine-tagged US11 was constructed by inserting an *Eco*RI/*Fsp*I US11-containing fragment from pRB4766 into *Eco*RI/*Hind*III (Klenow filled-in) digested pET-28c. Between the oligohistidine tag and US11 sequence are inserted amino acids LDSMGRLE.

Genomic DNA containing HSV1 VP16 was provided by P. O'Hare [3]. The plasmid pPO54 consists of the gene for VP16 inserted into the *Bam*HI site of pBS. An untagged VP16 construct was generated by firstly digesting pPO54 with *Bam*HI and inserting into the *Bam*HI site of pET-28c. VP16 was subsequently released from pET-28c by digestion with *Bam*HI/*Xho*I and inserted into the yeast vector pACT2 also cut with *Bam*HI/*Xho*I. VP16 was then released with an *Nco*I/*Xho*I digest and reinserted into the *Nco*I/*Xho*I site of pET-28a to allow expression of untagged VP16. The resulting fusion protein had an additional nineteen amino acids (MEAPGIRDPRSSFPYQPHP) at the N-terminus of VP16.

All pET constructs were sequenced at the 5' and 3' vector/insert junctions using T7 terminator and promoter primers. For pMAL-c2X constructs, the 5' vector/insert junctions were sequenced with the *mal*E primer.

Expression

The pET-28 constructs were expressed, harvested and lysed essentially as previously described [1]. The exceptions were induction of protein expression with 0.5 mM IPTG and lysis of bacteria expressing untagged US11 and VP16 into phosphate-buffered saline (PBS), pH 7.2. In addition, 0.1 % (v/v) NP-40 was interchanged with 0.1 % (v/v) Triton X-100.

MBP fusion proteins were expressed as above but grown in Luria broth containing 100 ug/ml ampicillin and 0.2 % (w/v) glucose. Protein expression was induced with 0.3 mM IPTG. Bacteria expressing MBP proteins were lysed by sonication in 20 mM Tris-HCl, pH 7.4, 200 mM NaCl, 1 mM EDTA, 1 mM DTT (MBP-resin buffer). All lysis buffers contained 1 mM PMSF and 5 µg/ml leupeptin.

Cells and viruses

Generation of virus stocks and titrations of HSV1 normal (CW1) and modified viruses were performed in Hep-2 cells using methods previously described [4, 5]. HSV1 US11-US12- deletion (R3631) [6], US11-US12+ deletion (R6604) and a US11 rescuant of R6604 (R6606) were provided by B. Roizman. R6604 is a partial US11 gene deletion mutant which does not express the US11 protein. For binding assays, Hep-2 cells were infected at a multiplicity of 5 pfu/cell and incubated for 24 h. Cell monolayers were washed twice with PBS, resuspended in PBS (1×10^6 cells/ml), freeze-thawed 3 times, sonicated for 15 s, before addition of 1% (v/v) NP-40 and incubation for 1 h at 4°C. The soluble fraction was harvested by centrifugation at 4°C (10000*g* for 15 min).

Binding assays

Oligohistidine-tagged uKHC (HisKHC) fragments were bound to His-bind resin as previously described [1], except beads were blocked for 1 h at room temperature with 5 % (w/v) BSA in binding buffer before charging with nickel sulphate. In addition, after incubation with HisKHC fragments and washing, beads were further incubated for 1 h at 4°C with 5 % (w/v) BSA in binding buffer, washed 2 x 20 volumes with wash buffer, followed by 1 x 20 volumes of low salt wash buffer (150 mM instead of 500 mM NaCl). Then 1 ml of either bacterial lysate containing untagged viral proteins, mock-infected or HSV1-infected Hep-2 soluble lysates were added. Beads were incubated overnight with rocking at 4°C, washed with 3 x 10 volumes of low salt wash buffer, and the bound proteins were eluted as previously described [1].

MBP-kinesin proteins were bound in a similar manner to amylose resin (NEB) except wash buffer was MBP-resin buffer. Blocking with 5% (w/v) BSA was done in PBS. After the second BSA blocking step the wash buffer was changed to PBS. Elution buffer was MBP-resin buffer plus 10 mM maltose.

Protein complexes and antibodies

Protein complexes were separated by SDS-PAGE and identified by immunoblotting as previously described [1]. Antibodies used included mouse monoclonal anti-US11 [7] (provided by B. Roizman), mouse monoclonal anti-VP16 [8] (LP1; provided by T. Minson) and rabbit polyclonal anti-HSV1 (Dako). Rabbit polyclonal antibody to HSV1 capsid protein VP5

(and VP23) was provided by G. Cohen and R. Eisenberg [9]. Monoclonal antibody to HSV1 gC was obtained from Chemicon International.

HSV infection of neurons

Preparation and culture of dissociated human fetal or rat neonatal DRG neurons, infection with HSV1, treatment with BFA, immunofluorescence, confocal and electron microscopy were performed as previously described [4, 5].

RESULTS

The present inventors have generated evidence for direct interaction between a HSV structural tegument protein US11 and ubiquitous kinesin. A direct role for US11 in HSV transport is supported by the abrogation of anterograde axonal transport of two HSV US11 deletion mutants in human dissociated DRG neurons. In addition, US11 was found to bind to the HSV tegument protein VP16 which may have a role in assembly of the virion tegument and anchoring of the virion-kinesin attachment.

Conventional kinesin is a heterotetramer consisting of two heavy and two light chains. The heavy chain has a three domain structure consisting of a motor domain, highly conserved among the kinesin superfamily, a stalk domain containing heptad repeats and a tail domain (Figure 1a). The present inventors have generated fragments of conventional human uKHC designated KHC stalk and KHC stalk/tail which overlap by one amino acid (Figure 1b). Along with essentially full-length kinesin light chain (KLC; 566 residues) and HSV US11 (161 residues), the fragments were tagged at the N-terminus with either a oligohistidine sequence (His) or maltose-binding protein (MBP). Successful expression of these fusion proteins in *Escherichia coli* was ascertained by purification with either Ni^{2+}-charged beads (His proteins) or amylose resin (MBP proteins; Figure 1c).

HisKHC fragments attached to Ni^{2+}-charged beads were incubated with a lysate of mock or HSV1-infected Hep-2 cells. Analysis of eluted complexes by immunoblotting with anti-HSV1 identified predominantly a 65 and 20 kDa viral protein preferentially bound to KHCstalk/tail (Figure 2a). These viral proteins were subsequently identified as VP16 (Figure 2b) and US11 (Figure 2c) using appropriate monoclonal antibodies. In the case of US11 there was some background binding to KHC stalk but binding to KHC stalk/tail was at least 10 fold greater for the same amount of KHC protein and thus deemed to be a specific interaction.

To establish whether US11 and/or VP16 bound directly to KHC, untagged forms of both were expressed in *E. coli*. Addition of untagged US11 or VP16 to KHC showed that US11 but not VP16 binds specifically to KHCstalk/tail both as His-tagged and MBP-tagged fusion proteins (Figure 3a & 3b). This also shows that the binding of US11 to KHC can occur in the absence of other viral proteins although it may well be modulated *in vivo* by viral or cellular factors. The light chain of kinesin appears not to bind directly to US11 since there was no detectable binding to MBP-KLC as compared to MBP-KHC stalk/tail (Figure 3b). Previous observations have also shown that a KHC stalk/tail fragment from sea urchin can bind to vesicles in the absence of KLC. It is therefore most likely that the viral protein US11 exploits a similar region to organelles for binding to KHC i.e. the tail domain. KLC may still modulate the interaction of US11 and KHC *in vivo* since it has been shown to be essential for axonal transport of cargo in *Drosophila*. In axons, uKHC antigen was also observed to colocalise with nucleocapsids coated with tegument and labelled for VP5 in axons of DRG neurons by dual immunogold labelling and freeze substitution-immunoelectron microscopy consistent with the biochemical findings.

The presence of VP16 coeluting with KHC when using viral lysates can be explained by the observation that VP16 binds directly to US11 (Figure 3c). This binding was observed with both native and recombinant VP16 (in the absence of other viral proteins). Therefore uKHC binds US11 which itself binds VP16. This VP16/US11 interaction may represent an important structural interaction in the tegument of the virus or may play a regulatory role in infected cells. VP16 has also been shown to bind to other tegument proteins including vhs and VP22, as well as to glycoprotein D. Therefore US11 may well interact with one or both of these tegument proteins.

US11 appears to be a multi-functional protein. It is a basic phosphoprotein which has RNA-binding activity, stably associates with 60S ribosomal subunits, has a role in post-transcriptional regulation of gene expression and localises to the nucleoli. The RNA-binding properties of US11 are dependent on the 20-24 RXP-repeats in the carboxy-half of the protein while the amino-half of US11 is required for transactivation of gene expression. It has been suggested that the N-terminal domain may be involved in transport and translation of mRNA from the nucleus to the cytoplasm. This amino domain may well also bind to kinesin. US11 is well

conserved between HSV1 and 2 (63% homology) with the greater variation at the N-terminus. There are no US11 homologues, however, in varicella-zoster virus or rabies virus.

To test the hypothesis that the US11–uKHC interaction mediated anterograde transport of HSV into the axons of fetal human and rat neonatal DRG neurons, the axonal transport of HSV nucleocapsids, and of those deleted in US11 were examined. The appearance and distribution of structural HSV protein antigens from the capsid (VP5), tegument (US11 and VP16) and envelope (glycoprotein C) and their anterograde transport into axons of dissociated human fetal and rat neonatal DRG was followed by fixation at serial time points over 24 hours and immunofluorescence/confocal microscopy as previously described. US11 was transported into axons of both human and rat DRG neurons *in vitro* with similar kinetics to that observed for major capsid protein VP5, in the presence or absence of brefeldin A (BFA; Figure 4a, b). The present workers have previously shown that incubation of BFA with HSV-infected neurons inhibits transport of glycoprotein C (gC) *via* the Golgi but not of capsid VP5 from the nucleus into axons. Further, BFA appears to disturb addition of some tegument proteins (VP16 and V22) to nucleocapsids, as illustrated with VP16 (Figure 4c & d), markedly inhibiting their transport into axons. Therefore, these results suggest that VP5 is co-transported anterogradely with US11 but not VP16 into axons in the presence of BFA, consistent with a direct role for US11 but not of VP16 in mediating transport.

Neither of the US11 deletion mutants of HSV1, R3631 (US11-) or R6604 (US11- 12-), showed normal transport of VP5 (or VP23) to the axon terminus of human and rat neurons, as illustrated with R6604 (Figure 4e). In neurons infected with control HSV and the US11 rescuant (R6606), VP5 (or VP23) antigen was diffusely distributed in the cytoplasm of the cell body and transported throughout the axon to the terminus by 24 hours (Figure 4f) as previously shown. With both deletion mutants, VP5 (or VP23) antigen was not detected in the axons of infected neurons (Figure 4e). With R6604 (US11-) there was a similar diffuse cytoplasmic distribution of VP5 to the control and rescuant. (With R3631, many neurons showed a slightly different phenotype with persistent accumulation of VP5 in the nucleus and perinuclear region rather than diffuse distribution in the cytoplasm). Anterograde transport of gC was unaffected by the deletions in both mutants

(Figure 4g, h). Transmission electron microscopy at late time points (26 hours) showed large numbers of enveloped virions in the extracellular space and in the cytoplasm of the cell body of neurons, concentrated around the Golgi. Unenveloped nucleocapsids were in variable abundance in the nuclei and sparsely scattered in the perinuclear cytoplasm often with concentrations adjacent to the Golgi (Figure 5). Thus the absence of US11 does not prevent transport across the nuclear membrane, normal envelopment and viral egress in the cell body i.e. there is no obvious functional disruption of the tegument. These findings also suggest that there are two separate mechanisms of viral egress, one via the cell body and the other via axon termini. The absence of US11 appears to disrupt the latter pathway between the perinuclear zone and the axon, possibly diverting viral egress via the cell body. The presence of US11 in the tegument, colocalisation with nucleocapsid VP5 in the axon (even in the presence of BFA) and the inability of two different HSV mutants deleted in US11 to be transported into axons is consistent with the hypothesis that the observed US11-uKHC interaction has a major role in mediating anterograde transport of nucleocapsids into and along axons. However, these results do not exclude cooperative effects with other HSV proteins.

This study raises many questions about the mechanisms of tegument formation, location of US11 in the tegument, the potential role of other kinesins and homologous interactions mediating the transport of other neurotropic viruses. Studies to map minimal binding regions of US11 and uKHC and identification of other cellular factors which facilitate or contribute to the interaction are currently in progress. Inhibition of this interaction by peptides and/or peptidomimetic analogues could provide a new strategy for antiviral treatment for this and other neurotropic viruses. Incorporation of this deletion into specifically attenuated live HSV vaccine candidates could also be useful to prevent clinical recrudescence.

It will be appreciated by persons skilled in the art that numerous variations and/or modifications may be made to the invention as shown in the specific embodiments without departing from the spirit or scope of the invention as broadly described. The present embodiments are, therefore, to be considered in all respects as illustrative and not restrictive.

Dated this twentieth day of July 1999

WESTMEAD HOSPITAL
THE UNIVERSITY OF SYDNEY
Patent Attorneys for the Applicant:

F B RICE & CO

REFERENCES

1. Diefenbach, R.J., Mackay, J.P., Armati, P.J. & Cunningham, A.L. The C-terminal region of the stalk domain of ubiquitous human kinesin heavy chain contains the binding site for kinesin light chain. *Biochemistry* 37, 16663-16670 (1998).

2. Cassady, K.A., Gross, M. & Roizman, B. The Herpes simplex virus US11 protein effectively compensates for the gamma 1 (34.5) gene if present before activation of protein kinase R by precluding its phosphorylation and that of the alpha subunit of eukaryotic translation initiation factor 2. *J. Virol.* 72. 8620-8626 (1998).

3. Hughes, T.A., La Boissiere, O'Hare, P. Analysis of functional domains of the host cell factor involved in VP16 complex formation. *J. Biol. Chem.* 274, 16437-16443 (1999).

4. Mikloska, Z., Sanna, P.P. & Cunningham, .AL. Neutralizing antibodies inhibit axonal spread of Herpes Simplex Virus type 1 to epidermal cells *in vitro J. Virol.* 73, 5934-5944 (1999).

5. Miranda-Saksens, M., Armati, P.J., Boadle, R.A., Holland, D.J. & Cunningham, A.L. Anterograde transport of herpes simplex virus type 1 in cultured dissociated human and rat sensory neurons. *J. Virol.* In press (1999).

6. Mavromara-Nazos, P., Ackermann, M. & Roizman, B. Construction and properties of a viable Herpes simplex virus 1 recombinant lacking coding sequences of the alpha 47 gene *J. Virol.* 60, 807-812 (1986).

7. Roller R.J. & Roizman, B. The Herpes simplex virus 1 RNA binding protein US11 is a virion component and associates with ribosomal 60S subunits, *J. Virol.* 66,3624-3632 (1992).

8. McLean, C., *et al.* Monoclonal antibodies to three non-glycosylated antigens of herpes simplex virus type 2. *J. Gen. Virol.* 60, 807-812 (1986).

9. Cohen, G.H. *et al.* Structural analysis of the capsid polypeptides of herpes simplex virus types 1 and 2. *J. Virol.* 34, 521-531 (1980).

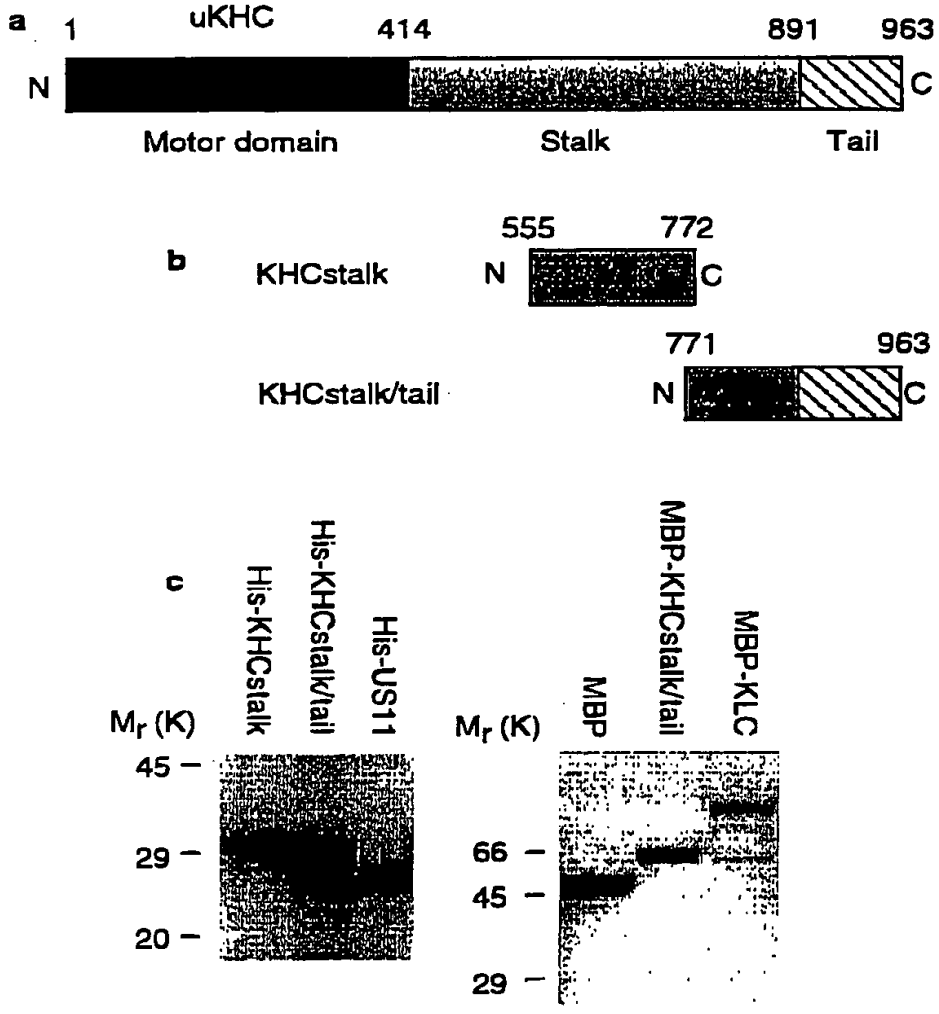

a
1
uKHC
414
891
963
N
C
Motor domain
Stalk
Tail
b
555
772
KHCstalk
N
C
771
963
KHCstalk/tail
N
C
c
His-KHCstalk
His-KHCstalk/tail
His-US11
Mr (K)
45
29
20
MBP
MBP-KHCstalk/tail
MBP-KLC
Mr (K)
66
45
29

Figure 1

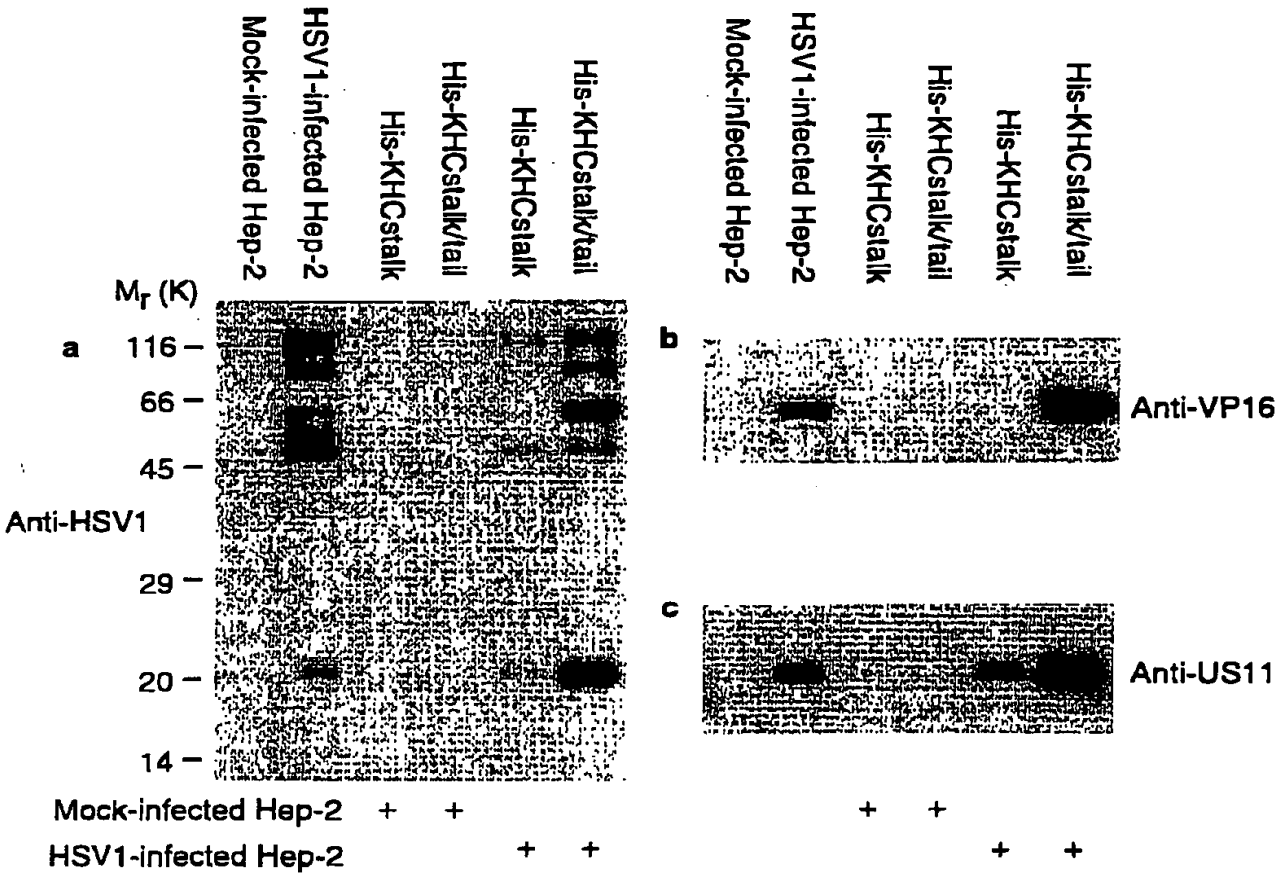

Mock-infected Hep-2
HSV1-infected Hep-2
His-KHCstalk
His-KHCstalk/tail
His-KHCstalk
His-KHCstalk/tail
M_r (K)
a
116
66
45
Anti-HSV1
29
20
14
Mock-infected Hep-2 + +
HSV1-infected Hep-2 + +
Mock-infected Hep-2
HSV1-infected Hep-2
His-KHCstalk
His-KHCstalk/tail
His-KHCstalk
His-KHCstalk/tail
b
Anti-VP16
c
Anti-US11
+ +
+ +

Figure 2

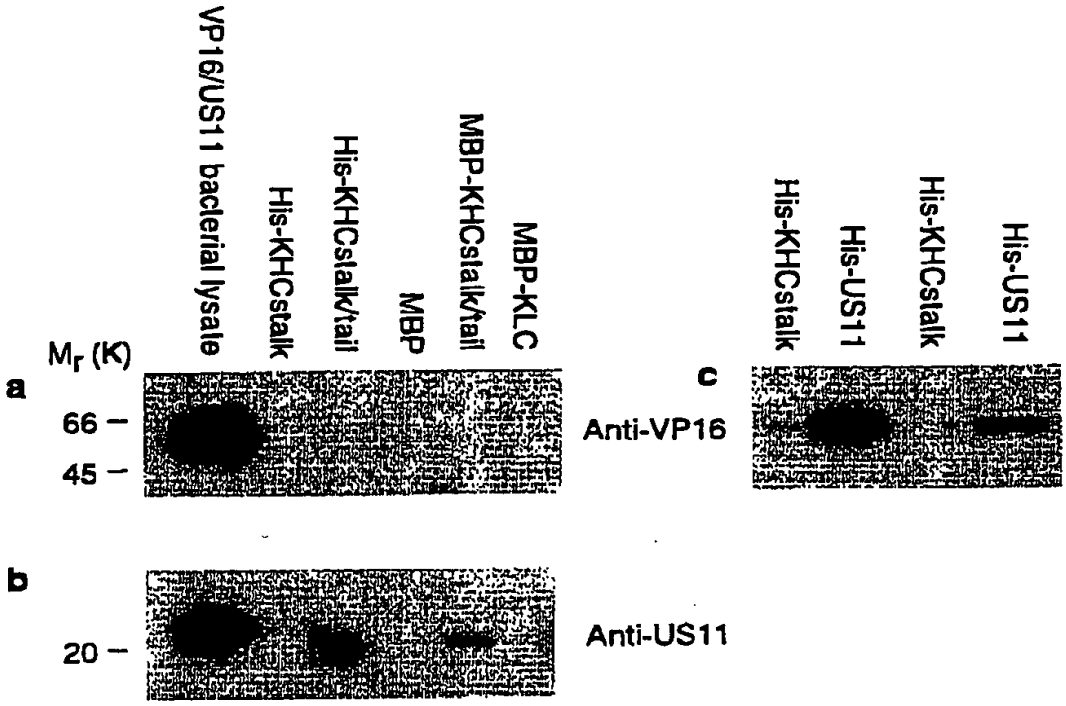

VP16/US11 bacterial lysate
His-KHCstalk
His-KHCstalk/tail
MBP
MBP-KHCstalk/tail
MBP-KLC
M_r (K)
a
66 –
45 –
b
20 –
Anti-US11
c
Anti-VP16
His-KHCstalk
His-US11
His-KHCstalk
His-US11

Figure 3

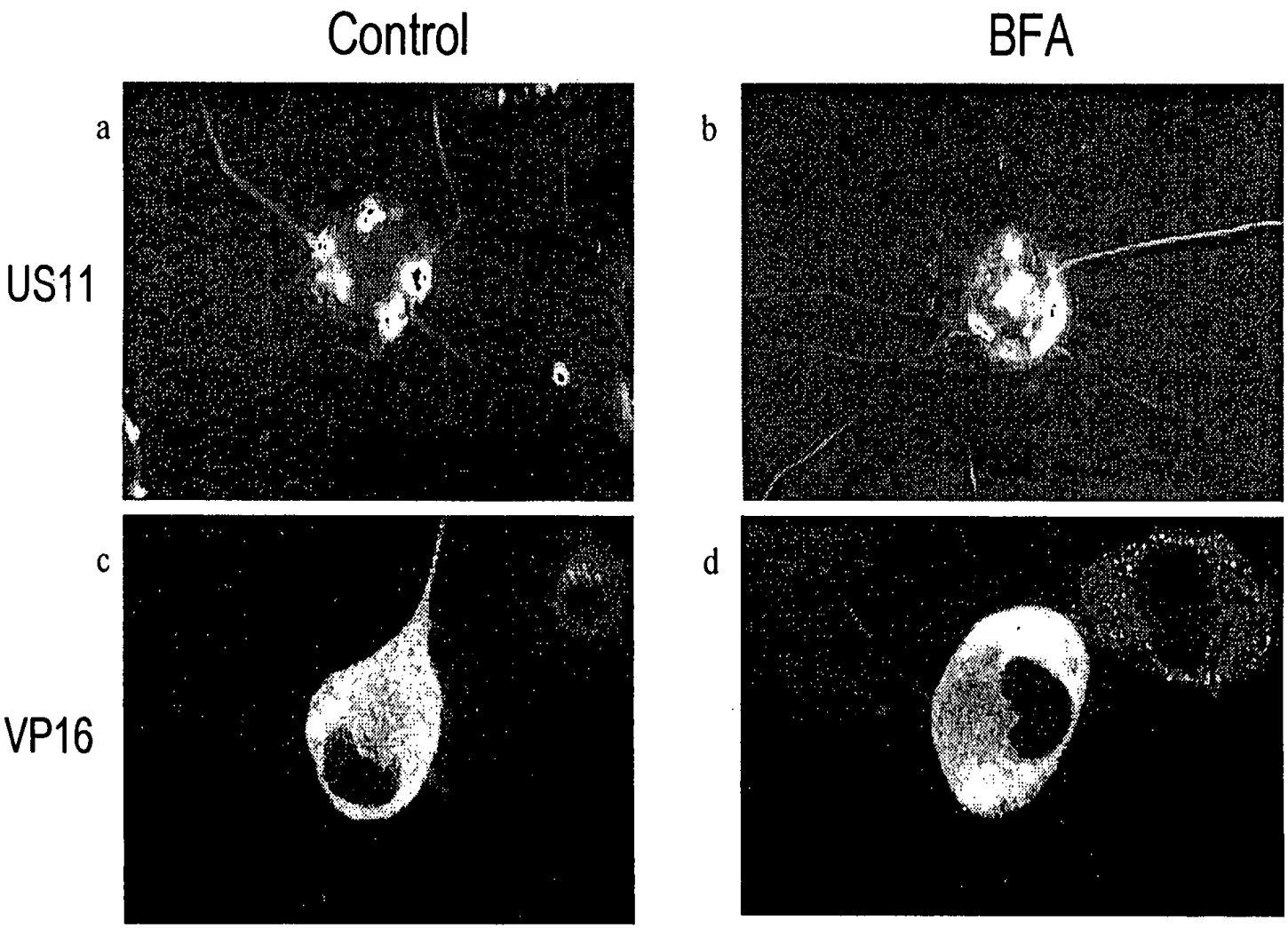


Figure 4a

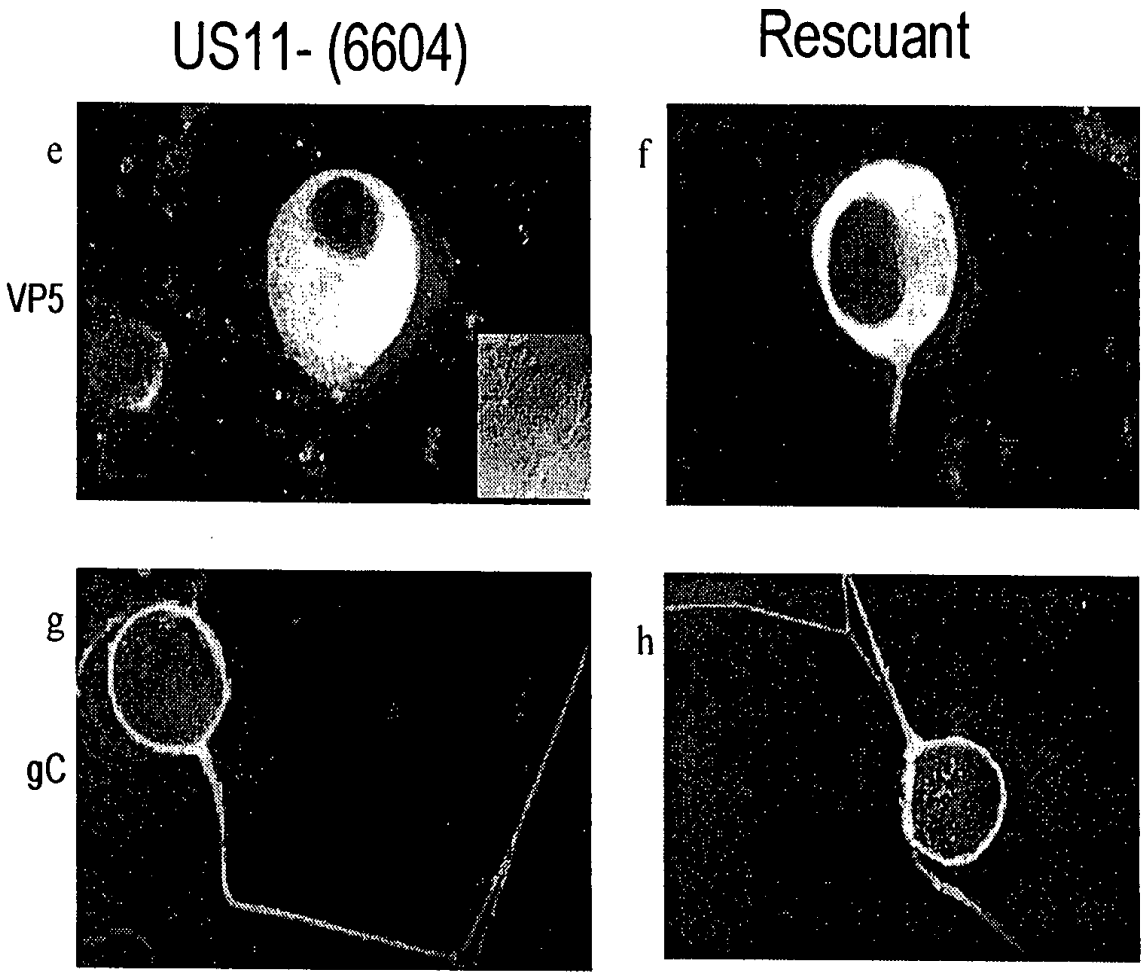
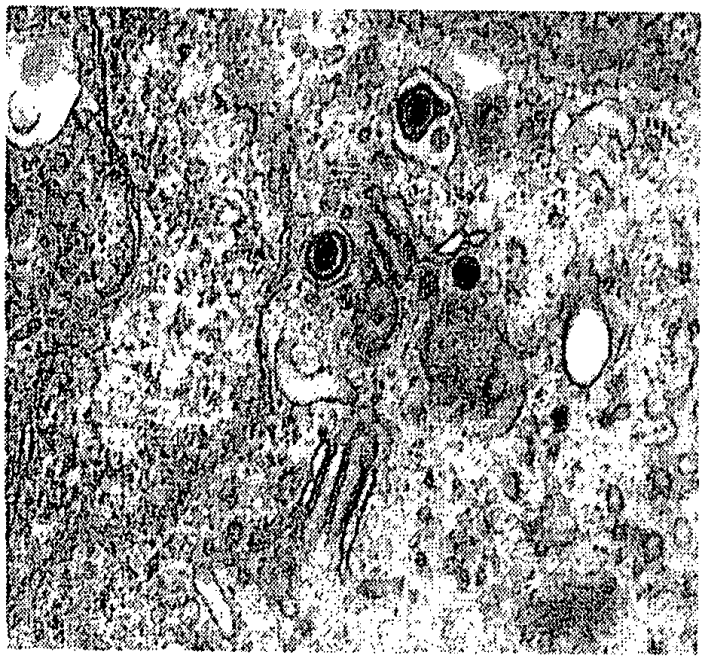


Figure 4b

9/5



A. Intracellular

B. Extracellular

9/9

Figure 5